RD-303
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: March 2, 1978. 001440

SUBJECT: EPA No. 464-LUA. Garlon 3A Herbicide. Application for registration of new chemical formulation. Caswell #882I.

FROM: Edwin R. Budd/Toxicology Branch/RD/OPP (WH-567)

TO: Robert Taylor/PM #25/RD/OPP (WH-567)

Action Requested: Registration of Garlon 3A Herbicide, Dow Chemical Company, containing 44.4% (W/W) Triclopyr (3,5,6-trichloro-2-pyridinyl-oxyacetic acid, as the triethylamine salt).

Recommendation: Toxicology Branch does not recommend in favor of registration of the subject formulated product at this time due, in part, to the weakly positive effect observed in the dominant lethal mutagenic assay in rats. Toxicology Branch is seriously concerned about the potential mutagenic significance of this finding and feels that additional mutagenic studies are fully warranted to fully assess the mutagenic potential of this material. Therefore, in addition to the 3 mutagenic studies already submitted, Toxicology Branch strongly recommends that a Heritable Translocation Test in Rodents be performed and that the results be submitted to EPA for evaluation. Until the results of this test are received and evaluated, Toxicology Branch will not recommend in favor of registration of this product. For additional discussion, see below under "Requirement for Heritable Translocation Test in Rodents".

Toxicology Branch defers to Chemistry Branch regarding possible nitrosamine problems in connection with this product. See below under "Deferral to Chemistry Branch Regarding Possible Nitrosamine Problem". A statement from Chemistry Branch is hereby requested.

The following submitted studies are classified as "Supplementary Studies" and therefore are not acceptable for the purpose of satisfying applicable toxicological requirements for registration of this subject product.

Studies performed on technical

*Acute Oral LD_{50}, Rats, Female
*Acute Oral LD_{50}, Rats, Male
Acute Oral LD_{50}, Cavies, Male
Acute Oral LD_{50}, Rabbits, Male and Female
*Primary Eye Irritation, Rabbits
*Primary Skin Irritation, Rabbits
*Teratology Study, Rabbits

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Studies performed on possible (?) metabolite (3,5,6-trichloro-2-pyridinol)

*Subacute Oral, Rats, 90-days

*required for registration of this product.

See reviews of individual studies for additional information on these studies.

The following additional studies shall be required for registration of this subject product. (They were not submitted in this package.)

Studies to be performed on formulated product

Subacute 21-day dermal study
Subacute 21-day inhalation study
Skin sensitization study
Photosensitization study (due to pyridine content)

Studies to be performed on technical

Heritable translocation mutagenic test in rodents (see below)

RPAR Criteria: None of the data reviewed in this submission triggers any RPAR criterion.

Requirement for Heritable Translocation Test in Rodents: Toxicology Branch recommends that the subject formulated product not ~~to~~ be registered at this time. This recommendation is based, in part, on the reported results of the dominant lethal assay in rats in which the test material produced a weak positive effect. See detailed review of this assay on pp. 16-22 of this review. In spite of negative results observed in two other submitted mutagenicity studies--the host-mediated assay in mice and the cytogenetic study in rats--Toxicology Branch feels that positive results in any dominant lethal assay warrant very serious further consideration.

It is to be noted that dominant lethal assays do not necessarily detect mutagenic (heritable) events directly and that

non-mutagenic reproductive events may also give positive results. (Positive reproductive effects were not observed, however, in the reproduction or teratogenic studies submitted for review.)

It should also be noted that dominant lethal assays are generally considered to be relatively insensitive tests that are capable of detecting only major chromosomal aberrations and/or other major genetic damage. For this reason, negative results in such studies have little toxicological meaning. Positive results, on the other hand, have great potential significance. Note also that the dominant lethal assay is an in vivo mammalian assay that assumes observed positive effects may possibly be due to chromosomal abnormalities produced in sperm. Potential chromosomal effects in sperm are obviously potentially heritable. The heritable nature of chromosomal effects is, by definition, an essential criterion for determination of mutagenic effect. The dominant lethal assay cannot, by itself, determine the heritability of effects. Such heritability is determined, however, in the Heritable Translocation Test in which an explicit genetic effect chemically induced in a parental generation is looked for in progeny generations. Therefore, in order to fully assess the mutagenic potential of the subject product, Toxicology Branch strongly recommends that a Heritable Translocation Test in Rodents be performed.

In the Translocation Test, mice or rats will be acceptable as the test species. Possible induced effects on sperm cell stages should be sampled throughout meiosis by careful scheduling of dosing and mating. Parental males should be treated and their male progeny subsequently mated to determine their semi-sterility or sterility. Cytogenetic analysis should be performed on males which are identified as sterile or semi-sterile to confirm the underlying chromosomal abnormality, if any.

Deferral to Chemistry Branch Regarding Possible Nitrosamine Problem: Toxicology Branch questions whether or not a nitrosamine problem may exist in connection with this product [redacted]. The active ingredient is Triclopyr (3,5,6-trichloro-2-pyridinyl-oxyacetic acid, as the triethylamine salt).

Information for which waiver is denied the manufacturing process is not included

Toxicology Branch defers to Chemistry Branch on this question and hereby requests a written statement from Chemistry Branch regarding this possible problem.

Background Information: Experimental Use Permit No. 464-EUP-46. EUP toxicity data reviewed by D. Ritter, 12/17/75. Triclopyr is a new chemical which has not previously been registered.

Summary of Submitted Toxicological Studies: All toxicity data submitted in support of this request for registration is summarized below.

Acute Toxicity - Technical

- Acute Oral LD_{50}, Rats, Female (=713 mg/kg). (Tox. Category III), Supplemental Study
- Acute Oral LD_{50}, Rats, Male (=713 mg/kg) (Tox. Category III), Supplemental Study
- Acute Oral LD_{50}, Cavies, Male (= 310 (236-407) mg/kg, (Tox. Category II), Supplemental Study.
- Acute Oral LD_{50}, Rabbits, Male & Female (= 550 (300-1000) mg/kg, (Tox. Category II), Supplemental Study
- Primary Eye Irritation, Rabbits (Slight corneal injury) (Tox. Category II), Supplemental Study
- Primary Skin Irritation, Rabbits (Slight redness (Tox. Category IV (?), Supplemental Study
- Acute Dermal LD_{50}, Rabbits = >1000 mg/kg (Tox. Category III,) Core Study - Minimum Data

Acute Toxicity - Formulation

Acute Oral LD_{50}, Rats, Female = 2140 (1540-2990) mg/kg Tox. Category III, Core Study - Minimum Data

Acute Oral LD_{50}, Rats, Male = 2830 mg/kg, Tox. Category III, Core Study - Minimum Data

Primary Eye Irritation, Rabbits - corneal damage at 7 days, Tox. Category I, Core Study - Minimum Data

Primary Skin Irritation, Rabbits - necrosis at 72 hours, Tox. Category II, Core Study - Minimum Data

Acute Dermal LD_{50}, Rabbits = >3980 mg/kg, Tox. Category III, Core Study - Minimum Data

Acute Inhalation LC_{50}, Rats = >0.8 mg/liter (recalculated) Tox. Category II, Core Study - Minimum Data

Subacute Toxicity - Technical

Subacute Feeding Study, Rats, 90 Days - NEL = 30 mg/kg/day, Core Study - Minimum Data

Teratology Study, Rabbits - (Negative for terata) Supplementary Study

Chronic Toxicity - Technical

Reproduction Study, Rats, 3-Generations - NEL ≥30 mg/kg/day, Core Study - Minimum Data

Mutagenicity Studies - Technical

Host-Mediated Assay, Mice - Negative, Core Study - Minimum Data

Mammalian Cytogenetic Study, Rats - Negative, Core Study - Minimum Data

Dominant Lethal Assay, Rats -- weak positive effect Core Study - minimum Data

Acute Toxicity - Possible Metabolite (?) 3,5,6-Trichloro - 2-pyridinol

Acute Oral LD_{50}, Rats, Male = 794 (709-889) mg/kg (Tox. Category III), Core Study - Minimum Data

Acute Oral LD_{50}, Rats, Female = 870 (758-1009) mg/kg (Tox. Category III), Core Study - Minimum Data

Acute Oral LD_{50}, Mice, Male = 380 (333-433) mg/kg (Tox. Category II), Core Study - Minimum Data

Acute Oral LD_{50}, Mice, Female = 415 (367-469) mg/kg Tox. Category II), Core Study - Minimum Data

Subacute Toxicity - Possible Metabolite (?) 3,5,6-Trichloro-2-pyridinol

Subacute Oral, Rats, 90 Days - (NEL = 0.1%), Supplementary Study.

Detailed Review of Submitted Studies:

The toxicity studies reviewed below are in Accession #229780 (Technical Information in Support of Application for New Registration of Garlon® 3A Herbicide, 4/29/77, Volume V, Human Safety, D.I. - D.12. pp. 31-295).

Acute Toxicity - Technical (D.2. pp. 36-43).

Olson, K. J., Toxicological Properties of 3,5,6-Trichloro-2-Pyridyloxy Acetic Acid, Biochemical Research Laboratory, Dow Chem Co., 7/27/67.

Test Material: 3,5,6-Trichloro-2-Pyridyloxy acetic acid, lot 1A10-1-109.

Acute Oral LD_{50}, Rats, Female = 713 mg/kg

Acute Oral LD_{50}, Rats, Male = 713 mg/kg

Acute Oral LD_{50}, Cavies, Male = 310 (236-407) mg/kg

Acute Oral LD_{50}, Rabbits, Male & Female = 550 (300-1000) mg/kg

Toxicity Category III (Rats)
Toxicity Category II (Cavies and Rabbits)

These studies are classified as Supplemental Studies (see below).

Single oral doses of test material (suspended in corn oil) to fasted animals. Five animals per group, 5 groups given 0.126 to 2.0 Gm/kg. Strains of test animals, ages or body weights not reported. Times of death, signs or necropsy results not reported. Length of observation period not reported.

Primary Eye Irritation, Rabbits

Unwashed Eyes - slight to moderate conjunctival redness lasting more than 7 days. Very slight corneal injury lasting less than 48 hours in one rabbit.

Washed Eyes - slight to moderate conjunctival redness lasting more than 7 days.

Toxicity Category II
Supplemental Study (see below)

Only 3 rabbits used. 100 mg of test material instilled into both eyes. 1 eye unwashed. Other eye washed for 2 minutes with tap water "within 30 seconds of instillation." Scoring system not given. Observations up to 7 days.

Primary Skin Irritation, Rabbits

Daily applications of 1-2 Gm of test material to bellies of 6 rabbits. Three animals received dry applications and 3 received applications with excess moisture. Intact and abraded test sites on each animal. Intact sites received 10 daily applications. Abraded sites received 3 daily applications. Three week observation period.

Dry, Intact - slight, sporadic exfoliation
Dry, Abraded - slight redness (for 24 hours, slight exfoliation
Moist, Intact - Negative for any effects
Moist, Abraded - slight redness and swelling (for 24 hours).

Toxicity Category IV (?)
Supplemental Study (see below)

The repeated applications, unusual protocol and lack of clear description of results (no scoring system given) make interpretation of this data difficult.

Acute Dermal LD_{50}, Rabbits >2000 mg/kg

Toxicity Category III
Core Study - minimum Data

2 Gm/kg of test material (plus 10 ml of water) applied to skin of 6 albino rabbits. Three rabbits had intact skin and 3 had abraded skin. 24 hours exposure. 14 day observation period. No untoward reactions or mortalities.

Acute Toxicity - Formulation (D.3., pp. 44-71)

Keeler, F.A., et. al., Acute Toxicological Properties of Experimental Herbicidal Formulation M3724 Containing Dowco 233, Toxicology Research Laboratory, Dow Chemical Co., 1/15/74.

Test Material: Experimental herbicidal formulation M3724 containing Dowco 233; Ref. No. 1-5302-48RVH; 3,5,6-trichloro-2-pyridyloxy acetic acid triethylamine [redacted]

Acute Oral LD_{50}, Rats, Female = 2440 (1540-2990) mg/kg

Acute Oral LD_{50}, Rats, Male = 2830 (no conf. lim) mg/kg

Toxicity Category III
Core Study - Minimum Data

Single oral doses of undiluted test material to fasted animals. 5 animals per group, 4 dosage groups for females ranged from 500-3980 mg/kg.

5 dosage groups for males ranged from 500-7950 mg/kg. 14 day observation period. Most deaths at ½ - 24 hours. Lethargy, narrowed eyes and piloerection (for first 7 hours); tremors and convulsions of 1 female at 2000 mg/kg and in 1 female at 3980 mg/kg.

Primary Eye Irritation, Rabbits

Unwashed Eyes - severe conjunctival irritation, moderate iritis and moderate to severe corneal injury in all 6 animals at 24 hours and persisting at least 7 days in most animals.

Washed Eyes - same as above. Washing had no effect.

Toxicity Category I (Corneal opacity not reversible within 7 days)

Core Study - Minimum Data

0.1 ml of test material instilled into both eyes of 6 rabbits. 1 eye unwashed, other eye washed for 2 minutes with tap water within 30 seconds of instillation. Readings at 24, 48 and 72 hours and at 7 days.

Primary Skin Irritation, Rabbits

0.5 ml of test material applied daily for 3 days to intact and abraded abdominal skin sites of 6 rabbits. Daily readings for 3 days.

Intact - slight to moderate erythema, slight edema and slight necrosis lasting at least 72 hours

Abraded - slight to moderate erythema, slight edema and slight to moderate necrosis lasting at least 72 hours.

Toxicity Category II (based on necrosis at 72 hours)

Core Study - minimum Data

Note - the repeated applications make interpretation difficult. It may be possible to change the Toxicity Category by repeating the study with 1 application.

Acute Dermal LD_{50}, Rabbits > 3980 mg/kg

Toxicity Category III
Core Study - Minimum Data

Undiluted test material applied to intact skin of 2 male and 2 female rabbits at 3980 mg/kg. 24 hour exposure. 14 day observation period. Body weights at 0, 1, 7 and 14 days. No mortalities.

Acute Inhalation LC_{50}, Rats > 5.34 mg/liter (3:20 dilution)

Toxicity Category II (recalculated to undiluted concentration of 0.8 mg/liter)

Core Study - Minimum Data

10 male and 10 female rats exposed to "aerosols of a 3 to 20 aqueous dilution" (a proposed use concentration) for 1 hour. The nominal aerosol concentration was calculated to be 5.34 mg/liter. Mean aerosol partiate size = 2.3 microns (99.9% of particles less than 7.0 microns). 14 day observation period. Gross necropsies. No signs of toxicity or irritation at any time. No mortalities. Gross necropsies were negative.

Subacute Toxicity - Technical

Subacute Feeding Study, Rats, 90 Days (D.4., pp. 72-116)

Humiston, C. G., et. al., 3,5,6-Trichloro-2-pyridyloxy acetic acid (Dowco 233 herbicide): 90-day dietary feeding

study in rats, Toxicology Research Laboratory, Dow Chemical Co., 1/29/75.

Test Material: Dowco 233 herbicide

14 Day Preliminary Range Finding Study: 300, 200, 100, and 30 mg/kg/day of Dowco 233 in diet. Decreased body weight gains at 300 and 200 mg/kg/day in males and females and at 100 mg/kg/day in males only. Mottled livers at necropsy. NEL = 30 mg/kg/day.

90 Day Study: 100,30, 10, 3 and 0 (controls) mg/kg/day of Dowco 233 incorporated into diet and given to a minimum of 10 male and 10 female Sprague-Dawley rats/dosage level. Mean body weights for males ranged from 242-252 Gm and for females from 197-204 Gm at beginning of study. Weekly body weights and food consumption. Hematology (packed cell volume, erythrocyte count, hemoglobin, white blood cell count and differential) at 30 days and at 84 days on 5 males and 5 females in control and 100 mg/kg/day groups. Urinalyses (pH, sugar, protein, Ketones, occult blood, bilirubin and specific gravity) at 30 days and at 84 days on 5 males and 5 females in control and 100 mg/kg/day groups. Clinical chemistries (serum urea nitrogen, alkaline phosphatase and SGPT) at time of sacrifice (87 days for males, 90 days for females) on 5 males and 5 females in all dosage groups. Gross necropsies, organ weights (heart, liver, kidneys, testes and brain) and organ/body weight ratios on all surviving rats. Histopathological examination (17 tissues) on 5 males and 5 females in control and 100 mg/kg/day group. Also, the eyes from 10 males and 10 females in control and 100 mg/kg/day groups were examined histopathologically.

Results: No mortalities were observed which were related to ingestion of test material. Body weights for males receiving 100 mg/kg/day were (generally) significantly lower ($p \leq 0.05$) than for controls - beginning at 13 days and continuing to end of study. Food consumptions for these same animals were (generally) lower over the same period. Hematology analyses and clinical chemistry data were essentially

negative. Gross necropsy data and histopathology data were essentially negative except as follows: males receiving 100 mg/kg/day had significantly decreased body weights and absolute liver weights and increased brain/body weight and kidney/body weights ratios. One female rat receiving 100 mg/kg/day died on day 57. Gross pathology revealed a tumor on the left kidney and increased size of the spleen. The tumor was histopathologically diagnosed as a nephroblastoma. The spleen showed evidence of extramedullary hematopoiesis. The occurrence of a single nephroblastoma in a single animal was not considered to be of particular concern (consultation with Dr. E. Long, Toxicology Branch on 10/7/77).

NEL = 30 mg/kg/day
Core Study - Minimum Data

Teratology Study, Rabbits (D.5. pp. 117-129)

Smith, F. A. et. al., The effect of Dowco 233 herbicide (3,5,6-trichloro-2-pyridyloxyacetic acid) on the developing embryo and fetus of pregnant rabbits, Toxicology Research Laboratory, Dow Chemical Co., 2/13/75.

Test Material: Dowco 233 herbicide, GHC 25-1-47.

Preliminary Range Finding Study: Dowco 233 orally administered as a corn oil suspension to 3 non-pregnant female rabbits/dosage level for 13 days. Dosage levels were 300, 200, 100, 50 and 0 (control) mg/kg/day. Dosage forms were adjusted so that the proper mg/kg was administered in a volume of 1 ml/kg. Following cessation of dosing, the animals were observed for an additional 11 days. 2/3 animals died at 300 mg/kg/day and 1/3 at 200 mg/kg/day. Maximum tolerated dose determined to be 100 mg/kg/day.

Teratology Study: Dowco 233 orally administered as a corn oil suspension to 15 bred (hand-mated on day 0) female New Zealand rabbits per dosage levels daily from days 6 to 18 of gestation. Three dosage levels were used--100, 50, and 25 mg/kg/day. 25 control

rabbits were treated similarly. Dosage forms were adjusted so that the proper mg/kg was administered in a volume of 2 ml/kg. Frequent observations and body weights. All animals were sacrificed and Caesarean sectioned on day 29. The number of corpora lutea in each ovary was determined; liver weights were determined; and the number and position of live, dead and resorbed fetuses were determined. Body weights, crown-rump measurements and sexes of fetuses were determined. Following external examinations, 1/3 of each litter was examined for soft tissue anomalies. All fetuses were examined for skeletal anomalies.

Results: Mortalities were extremely high. Applicant reported 31%, 57% and 53% mortalities in the 25, 50 and 100 mg/kg/day groups respectively. Examination of the data presented, however, indicates 27%, 80% and 47% mortalities in the 25, 50 and 100 mg/kg/day groups, respectively. In addition, 44% of the control animals appear to have died. The data is extremely difficult to understand and interpret because much of it is inconsistent and many animals cannot be accounted for. Times of death and specific animals that died are not reported. No data on number of fetuses, whether absorbed or not, terata or any other reproductive or teratogenic parameters were reported for animals that died. Data that was presented is as follows: 14/25 control animals; 11/15 25 mg/kg/day animals; 3/15 50 mg/kg/day animals. Teratogenic effects were not observed in these animals. Statistical evaluations provided are of little value due to the inconsistencies described above. Data on individual animals is lacking. and 6/15 100 mg/kg/day animals

✓ This study is classified as a Supplementary Study for the reasons described above.

Chronic Toxicity - Technical

Reproduction Study, Rats, 3-Generations (D.6, pp. 130-194)

Three-generation reproduction study in rats [on] Dowco 233, Final Report, Litton Bionetics, Inc., LBI Project No. 2528, 11/4/76.

Test Materials: Dowco 233, AGR 134832 (for first part of test) and Triclopyr, 134832 (for last part of test) - both materials presumably identical.

Protocol: Test material incorporated into diets of Sprague-Dawley rats, 35-40 days old at beginning of study, at 0 (control), 3, 10 and 30 mg/kg/day. 11-12 males and 23 females/dosage level. After 56 days of feeding, these animals (P_1) were mated to produce the F_1 generation. F_1 animals were weighed, examined and observed. At day 4, litters were reduced to 10 pups--extra pups were discarded. Following feeding of F_1 animals for 100 days, they were mated to produce the F_2 generation, which was similarly treated to produce the F_3 generation. F_3 animals were sacrificed at weaning time. Gross necropsies on all animals that died and on F_2 adults and F_3 weanlings. Histopathological examination (38 tissues) on F_2 adults (5 rats/sex from control and 30 mg/kg/day groups) and on all gross lesions. Daily observations, weekly body weights and food consumptions until breeding. Body weights on females on days 0, 7, 14 and 20 of gestation and on days 0, 7, 14 and 21, of lactation. Other standard reproductive parameters also noted. Note that there is only 1 litter/generation in this study.

Results: No effects due to test material were observed in any of the parameters described above at any of the dosage levels. NEL $\geq$ 30 mg/kg/day.

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Mutagenicity Studies - Technical

Host-Mediated Assay (D.7., pp. 195-218)

In vitro and subacute in vivo host-mediated assay for mutagenesis, Final Report, Litton Bionetics Inc., LBI Project No. 2421, 11/6/73.

Test Material: Dowco 233, BRL #702.

Indicator Organisms: Salmonella TA-1530, Salmonella G-46 and Saccharomyces D-3.

Host: Charles River ICR random bred male mice.

Direct in vitro assays: TA-1530 and G-46 plated directly with discs containing 0.1 ml of saturated solution of test material and observed for revertants. Positive control was ethylmethylsulfonate (EMS). D-3 treated with test material ~~were~~ 4 hours at 30°C and then plated for determinations of surviving total population and recombinant red sectors. (D-3 yeast cells were treated with a concentration of test material previously determined to produce 50% survival) Positive control was EMS.

Host-Mediated Assay: Test material orally administered to groups of 10 mice/dosage level. 3 dosage levels--0.7, 7.0 and 70.0 mg/kg. Positive control for Salmonella strains was dimethylnitrosamine (DMN) and for Saccharomyces was EMS. In acute tests, indicator organism was intraperitoneally injected immediately after administration of test material. In subacute tests, test material was administered 5 times (at 24 hour intervals) followed by injection of indicator organisms ½ hour after last administration. Four hours after injection of indicator organisms, animals were killed. Peritoneal fluid was recovered, diluted and plated as in vitro assays. Salmonella revertants were reported as MF_t/MF_c; Saccharomyces recombinants as MR_t/MR_c.

Results: The test material induced no significant increases (over negative controls) in mutant or recombinant frequencies in the in vitro or in vivo studies at the dosage levels tested.

Core Study - Minimum Data

Mammalian Cytogenetic Study (D.8. pp. 219-227)

Acute and subacute in vivo cytogenetic study in rats, Final Report, Litton Bionetics Inc., LBI Project No. 2421, 9/14/73.

Test Material: Dowco 233, BRL #702

Acute study: Test material was orally administered to groups of 5 rats (Sprague Dawley, males, 10-12 weeks old) at dosage levels of 0.7, 7.0 and 70.0 mg/kg. A positive control group (triethylene melamine, TEM) and 2 negative control groups (corn oil and saline) were also utilized. Groups of animals were sacrificed and examined as described below at 6, 24 and 48 hours after administration.

Subacute study: Test material was orally administered daily for 5 days to groups of 5 rats at dosage levels of 0.7, 7.0 and 70.0 mg/kg. A negative control group (corn oil) was also utilized. All animals were sacrificed at 5 days after the last administration of test material.

Cytological examination procedure: Bone marrow cells were arrested in C-metaphase, by the intraperitoneal injection of colcemid and then examined histologically. Chromosomes were counted and scored for aberrations. 50 spreads per animal were scored. A mitotic index (number of cells in mitosis/number of cells observed) was also calculated..

Results: No cells with chromosomal aberrations were observed in test groups or in negative control groups in either the acute or subacute study. Chromosomal aberrations were observed in the positive control group. Mitotic indices were within normal limits.

✓ Core Study - Minimum Data

Dominant Lethal Assay, Rats (D. 9., pp. 228-251)

Dominant Lethal Assay for Mutagenesis, Final Report, Litton Bionetics Inc., LBI Project No. 2421, 11/19/73.

Test Material: Dowco 233, BRL #702

Test Animals: Rats, Sprague Dawley CD strain, males and females, 10-12 weeks old, 250-320 Gm.

Protocol: Test material was orally administered, by gastric intubation, daily for 5 days to groups of 10 male rats at dosage levels of 0.7, 7.0 and 70.0 mg/kg. Triethylene melamine (TEM)$_x$ was utilized as a positive control (single intraperitioned injections of 0.30 mg/kg to 10 male rats). Two negative control groups, each containing 10 male rats, were also utilized (corn oil and saline). Following treatments, the males were sequentially mated to 2 untreated females per week for 7 weeks. Females were killed at 14 $\pm$ 2 days after mating. At necropsy, the uterus was examined for early fetal deaths, late fetal deaths, total implantations per uterine horn and number of corpora lutea. From this data base, the following 8 parameters were calculated and evaluated:

1. fertility index
2. total number of implantations
3. total number of corpora lutea
4. preimplantation losses
5. dead implantations
6. proportion of females with 1 or more dead implantations
7. proportion of females with 2 or more dead implantations
8. dead implants/total implants

Each parameter for each week was analyzed and evaluated independently.

Results: Results for the 8 parameters (described above) are given below:

1. fertility index - decrease in the number of pregnant females/number of mated females at the 7.0 mg/kg and 70.0 mg/kg dosage levels during week 1 (on). The significance of this finding is difficult to evaluate since the animals were mated so soon after administration of the test material and may have been suffering from malaise.

2. total number of implantations - essentially negative for effects due to the test material.

3. total number of corpora lutea - essentially negative for effects due to the test material.

4. preimplantation losses - essentially negative for effects due to the test material.

5. dead implantations - there was a trend toward increased average resorptions (dead implants) per pregnant female at the 7.0 mg/kg and 70.0 mg/kg dosage levels during much of the 7 week testing period. Although only occasionally statistically significant, the trend was clear. Statistically significant increases were observed in 7.0 mg/kg animals at week 4 and in 70.0 mg/kg animals at weeks 5 and 7.

~~6. proportion of females with 1 or more dead implantations - there was a tendency toward increased proportions at the 7.0 mg/kg and 70.0 mg/kg dosage levels during much of the 7 week testing period. A statistically significant increase was observed in 70.0 mg/kg animals at week 7.~~

Average Resorptions (Dead Implants) per Pregnant Female

Week	Negative Control	Test 0.7 mg/kg	Test 7.0 mg/kg	Test 70 mg/kg	Positive Control
1	.36	.07	.19	.60	3.67[b]
2	.25	.44	.89	.54	4.08[b]
3	.67	.34	.75	.83	2.89[a]
4	.17	.63	1.38[a]	.69	3.58[b]
5	.23	.44	.57	.89[a]	.45
6	.27	.46	.74	.50	.62
7	.30	.63	.53	1.08[a]	.42

[a]Significantly higher than negative control in 1 - tailed test, $p < 0.05$

[b]Significantly higher than negative control in 1 - tailed and 2 - tailed test, $p < 0.01$

evaluations by t-test

6. proportion of females with 1 or more dead implantations - there was a trend toward increased proportions at the 7.0 mg/kg and 70.0 mg/kg dosage levels during much of the 7 week testing period. A statistically significant increase was observed in 70.0 mg/kg animals at week 7.

Proportion of Females with One or More Dead Implantations

Week	Negative Control	Test 0.7 mg/kg	Test 7.0 mg/kg	Test 70 mg/kg	Positive Control
1	.22	.07	.19	.50.	.74[e]
2	.25	.25	.36	.34	.79[e]
3	.54	.29	.19[c]	.48	.48
4	.12	.25	.32	.32	.65[e]
5	.23	.32	.38	.42	.23
6	.27	.28	.20	.43	.28
7	.24	.32	.18	.62[d]	.30

[c]Significant lower than negative control at P <0.05

[d]Significantly higher than negative control at P <0.05

[e]Significantly higher than negative control at P<0.01

evaluations by chi-square test

7. proportion of females with 2 or more dead implantations - there was a trend toward increased proportions at the 7.0 mg/kg and 70.0 mg/kg dosage levels during much of the 7 week testing period. Statistically significant increases were observed in 7.0 mg/kg animals at week 4 and in 70.0 mg/kg animals at week 5.

Proportion of Females with Two or More Dead Implantations

Week	Negative Control	Test 0.7 mg/kg	Test 7.0 mg/kg	Test 70 mg/kg	Positive Control
1	.08	.00	.00	.10	.67[e]
2	.00	.13	.18	.20	.79[e]
3	.07	.07	.13	.18	.48[d]
4	.06	.19	.32[d]	.16	.65[e]
5	.00	.13	.07	.30[d]	.12
6	.00	.19	.20	.08	.23
7	.06	.19	.12	.16	.12

[d]Significantly higher than negative control at $P < 0.05$

[e]Significantly higher than negative control at $P < 0.01$

chi-square

8. dead implants/total implants - there was a trend toward increased ratios at the 7.0 mg/kg and 70.0 mg/kg dosage levels during much of the 7 week testing period. Statistically significant increases were observed in 7.0 mg/kg and 70.0 mg/kg animals at week 5.

Dead Implants/Total Implants

Week	Negative Control	Test 0.7 mg/kg	Test 7.0 mg/kg	Test 70 mg/kg	Positive Control
1	.04	.01	.02	.07	.45[b]
2	.03	.04	.08	.05	.45[b]
3	.06	.03	.08	.07	.37[b]
4	.02	.05	.12	.06	.37[b]
5	.02	.04	.06[a]	.08[f]	.05
6	.03	.04	.06	.05	.06
7	.03	.05	.05	.09	.04

[a]Significantly higher than negative control in 1-tailed test, $P < 0.05$

[f]Significantly higher than negative control in 1-tailed and 1-tailed test, $P < 0.05$

[b]Significantly higher than negative control in 1-tailed and 1-tailed test, $P < 0.01$

evaluations by t-test

The above results (average resorptions per pregnant female, proportion of females with one or more dead implantations, proportion of females with two or more

dead implantations, and dead implants/total implants) indicate a weak positive effect produced by Dowco 233 in this dominant lethal assay.

This study is classified Core Study - Minimum Data.

Acute Toxicity - Possible Metabolite (?), 3,5,6-Trichloro-2-pyridinol

Acute Oral LD_{50}, Rats, (D. 10., pp. 252-256).

Gerbig, C. G. and Emerson, J. L., Oral median lethal dose (LD_{50}) determination of 3,5,6-trichloro-2pyridinol in rats, Dept. of Pathology and Toxicology, Dow Chemical Co., 6/5/70.

Test Material: 3,5,6-trichloro-2-pyridinol, Ref. 238-11-112.

Acute Oral LD_{50}, Rats, Male = 794 (709-889) mg/kg

Acute Oral LD_{50}, Rats, Female = 870 (758-1009) mg/kg.

(Toxicity Category III)

Core Study - Minimum Data

Test material suspended in 0.5% hydroxypropyl methyl cellulose (Methocel). Following preliminary range finding studies, single oral doses of test material to fasted animals (Sprague Dawley rats, 78-96 Gm) 10 males and 10 females per dosage level. 5 dosage levels ranging from 794 to 1260 mg/kg. 14 day observation period. Gross necropsies. All deaths at 10 minutes to 4 hours.

Signs were flaccid paralysis with dyspnea and slight hypersalivation (within 5 minutes in all treated animals). The severity of these signs were uniform in all rats. Rats that died developed a rigor mortis like rigidity of the whole body within seconds following death. Survivors returned to normal by 24 hours. Gross necropsies were negative.

Acute Oral LD_{50}, Mice (D. 11., pp. 257-261)

Gerbig, C. G. and Emerson, J. L., Oral median lethal dose (LD_{50}) determination of 3,5,6-trichloro-2-pyridinol in mice, Department of Pathology and Toxicology, Dow Chemical Company, 6/10/70.

Test Material: 3,5,6-trichloro-2-pyridinol, Ref. 238-11-112.

Acute Oral LD_{50}, Mice, Male = 380 (333-433) mg/kg

Acute Oral LD_{50}, Mice, Female = 415 (367-469) mg/kg

(Toxicity Category II)

Core Study - Minimum Data

Test material suspended in 0.5% Methocel. Following preliminary range finding studies, single oral doses of test material to fasted animals (Swiss mice, Cox strain, 15-19 Gm). 15 males and 15 females per dosage level. 5 dosage levels ranging from 354 to 891 mg/kg. 14 day observation period. Gross necropsies. All deaths at 2 minutes to 2 hours. Signs were tremors followed by flacid paralysis and dyspnea in all treated mice (within 2 minutes in higher dosage level groups and within 5 minutes in lower dosage level groups). Exophthalmia in a few mice. Males appear to be more severly affected. Mice that died developed a rigor mortis like rigidity of the whole body within seconds following death. Survivors normal by 24 hours. Gross necropsies were negative.

Subacute Toxicity - Possible Metabolite (?) 3,5,6-trichloro-2-pyridinol

Subacute Oral, Rats, 90 Days (D. 12., pp. 262-295).

Results of 90-day dietary feeding studies of 3,5,6-trichloro-2-pyridinol in rats, Biochemical Research Lab., Dow Chemical Co., 7/9/64.

Test Material: 3,5,6-Trichloro-2-pyridinol, OL2B5-16-30

Protocol: Test material was administered in the diet to 51 day old rats (mean weights 120 Gm and 140 Gm for females and males respectively) for 90 days at dosage levels of 0.0 (controls), 1.0, 0.3, 0.1, 0.03 and 0.01 percent. 10 rats/sex/dosage level. Observations included body weights (2 times/week for first 4 weeks and weekly thereafter), appearance, behavior, and food consumption (for first month). Terminal hematology (hematocrit, hemoglobin, leukocyte count and differential) on 5 female rats at control, 1.0 and 0.3 percent levels. Gross necropsies and organ weights on 7 organs. Histopathological examination on 24 tissues and organs and bone marrow smears on 5 males and 5 females in control and 1 percent groups only. Terminal blood chemistries (serum urea nitrogen and alkaline phosphatase) on unknown number of animals.

Results: No evidence of adverse effects due to the test material in any of the parameters described above at 0.01, 0.03 or 0.1 percent dosage levels. At 1 percent, both females and males exhibited decreased growth rates and evidence of diuresis during the entire study. Decreased food consumption in females but not in males. Increased organ/body weight ratios for kidney, spleen, testes and brain were possibly due to decreased body weights. Increased liver/body weight ratios were dose related and may be related to the test material. Dry, bloody noses observed during first month. Other parameters were negative. At 0.3 percent, liver/body weight ratios were significantly increased in females. Diuresis during entire testing period in both females and males. Gross pathology presented was insufficient and inadequate.

This study is classified as a Supplementary Study for the following reasons:

1. strain of rats not reported
2. no urinalyses were performed

3. hematology only on female rats and only at termination of study

4. insufficient number of blood chemistries performed on unknown number of animals per group

5. gross necropsy data (other than organ weights and ratios) was insufficient and inadequate

6. histopathological examination performed on insufficient number of animals.

7. inconsistencies and carelessness (?) in reporting of data.

Edwin R. Budd

Edwin R. Budd
Toxicology Branch

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